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Histone variants and sensing of chromatin functional states

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An extreme case of chromatin remodelling is the genome-wide exchange of histones with basic non-histone DNA-packaging proteins that occurs in post-meiotic male germ cells. The scale of this genome reorganization is such that chromatin needs to undergo a prior "preparation" for a facilitated action of the factors involved. Stage-specific incorporation of specialized histone variants, affecting large domains of chromatin, combined with histone post-translational modifications accompany the successive steps of the male genome reorganization. Recently, it has been shown that a testis-specific H2B variant, TH2B, one of the first identified core histone variants, replaces H2B at the time of cells' commitment into meiotic divisions and contributes to the process of global histone removal. These investigations also revealed a previously unknown histone dosage compensation mechanism that also ensures a functional interconnection between histone variant expression and histone post-translational modifications and will be further discussed here.

Keywords: acetylation, arginine methylation, crotonylation, histone chaperone, histone degradation

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Introduction

Spermatogenesis is the commitment of progenitor spermatogenic cells, spermatogonia, into a cell differentiation process involving first the meiotic divisions of spermatocytes, then the post-meiotic differentiation of spermatids. Both are associated with extensive cellular and nuclear reorganizations.[1] Spermatids later eventually give rise to the mature spermatozoa, which are capable of leaving and surviving outside the parent organism. One remarkable characteristic of sperm differentiation is a large-scale involvement of chromatin modifying determinants such as histone variants and histone post-translational modifications (PTMs).[2,3] While histone variants can directly alter the structure of nucleosomes, due to the divergence of their sequence compared with canonical histones,[4,5] histone PTMs could constitute a basis for region-specific genome signposting, implying the recruitment of specific factors that activate or repress transcription. Additionally, histone PTMs can also directly modify nucleosome stability by altering the properties of structurally strategic histone amino acids.[6]

These chromatin-modifying mechanisms are largely involved in spermatogenic differentiation. Meiotic recombination requires loci-specific alterations of chromatin in spermatocytes, a process that occurs simultaneously to a chromosome-wide transcriptional silencing of sex chromosomes, known as meiotic sex chromosome transcriptional inactivation (MSCI).[7] In pachytene spermatocytes, during MSCI, X, and Y chromosomes undergo an extensive replacement of their replication-dependent H3s by H3.3 and a chromosome-wide phosphorylation of histone H2A variant H2A.X.[7,8] In early post-meiotic cells, known as round spermatids, although the sex chromosomes remain largely transcriptionally inactive, a number of sex-linked genes, encoding factors with critical post-meiotic roles, becomes reactivated.[3,9] When round spermatids differentiate into elongating spermatids, a general transcriptional shutdown precedes the global histone hyperacetylation and histone removal.[2,10] During all of

these processes, spermatogenic cells seem to synergistically use histone variants and histone PTMs to comply with the required need for a large-scale action on chromatin.[11]

Spermatogenic cells express almost all histone variants including unique testis-specific members.[12] However, although some of these variants were shown to cover regions at a chromosome scale, until recently, none had been shown to replace its canonical counterparts at a whole-genome scale. In our recently published report we observed that the *Th2b* gene is activated in spermatocytes while the corresponding protein gradually replaces H2B, finally covering the whole genome.[13] This event is truly remarkable, since we discovered that a single histone variant can almost entirely replace its canonical counterparts. With this respect, TH2B could clearly be considered as a particular histone variant with a genome-wide action potential. Interestingly, the generation of TH2B-less fertile male mice revealed that this genome-wide role of TH2B can be fully compensated. Indeed we found that the absence of TH2B generates a compensation mechanism involving both the activation of the *h2b* gene and the occurrence of specific histone PTMs.[13]

TH2B seems therefore able (1) to coordinate the expression of *h2b* genes in general, and (2) to communicate with the histone PTM machinery. Here, based on our recently published work on TH2B[13] and our analysis of existing data in the literature, we will discuss how histone genes could communicate with each other and how the histone PTM machineries could target specific structurally critical positions in histones.

Cross-talk Between Histone Genes: Histone Dosage Compensation

A transcriptomic and proteomic analysis of TH2B-less spermatogenic cells revealed an upregulation of various *h2b* genes at both the mRNA and protein levels. Ultrahigh performance LC-MS (ULHMC-MS) detected the accumulation of at least six H2B canonical isoforms showing variable degrees of induction, ranging from 2.5 to 7-folds over their normal expression in wild type spermatogenic cells. This observation indicates that, during normal spermatogenesis, the activation of the *Th2b* gene and the replacement of H2B by TH2B is required for the downregulation of the *h2b* gene expression. In other words, a sensing mechanism is likely adjusting the expression of *h2b* genes to maintain an adequate amount of H2B in chromatin.

The compensatory response of *h2b* genes to the absence of TH2B is reminiscent of the situation observed with H1 isoforms, more precisely when the testis-specific H1 variant H1T is knocked out. In pachytene spermatocytes, *h1T* is expressed after *Th2b*, and could constitute up to 50% of linker histones in the following spermatogenic cells.[14] The knockout of the *h1T* gene did not affect in any way spermatogenic cell differentiation.[15-17] Moreover the loss of H1T was compensated as no reduction of the total amount of linker histone could be detected.[15,16] Indeed genes encoding other linker histones were upregulated to maintain a correct amount of H1.

In these two examples with H1 and H2B variants, canonical histones compensate for a downregulation of their corresponding variant. Therefore, a sensing mechanism seems to adjust the expression of each histone and its respective variants to reach the required amounts of histones per nucleosome. This sensing mechanism is probably specific for each class of histones, since the response to TH2B or H1T downregulation is limited to the *h2b* and *h1* genes respectively, and do not affect other histone-encoding genes. Interestingly, within each class of histones, the variant genes located in a separate genomic region are also part of this "sensing mechanism," i. e. *Th2b* communicates with *h2b*s and *h1T* with *h1*s.

How could *h2b* genes sense the lack of TH2B in chromatin? What could be the nature of this sensing mechanism and how is it measuring the histone levels? Answers to these questions can be speculated from a few reports describing a general sensing mechanism of non-assembled free histones.

During DNA replication, any excess of free histones, which could be very toxic to cells, is rapidly eliminated. The mechanism uncovered in yeast involves the kinase activity of Rad53, which in contrast to the cases discussed here, acts on the total histones dosage.[18,19] A way to establish a histone class-specific sensing mechanism could be to involve histone chaperones that show some degree of specificity for various histone classes and histone variants.[20]

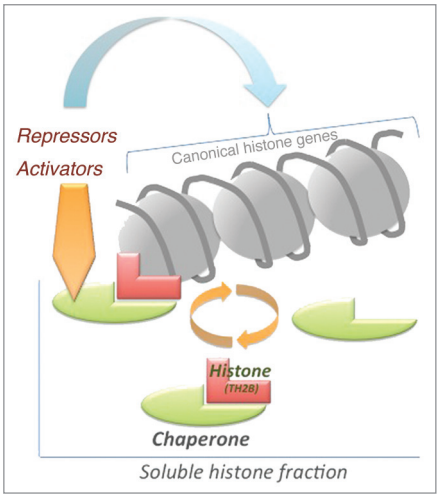

Figure 1. A model for chaperone-mediated sensing of chromatin states. Chaperone-histone interaction upon nucleosome assembly recruits transcriptional activators/repressors modifying the state of gene expression as a function of the amount of assembled histone.

It has been proposed that in human replicative somatic cells, the pool of free histones does not exceed 0.1% of total histones.[21,22] Therefore, the majority of histone chaperones, even in replicative cells, are present in their histone-free form.[23] In comparison to dividing cells, spermatocytes and spermatids could have even less free histones since these cells do not replicate their DNA. Therefore, any excess of free histones could be stored under a chaperon-bound form, which could facilitate their degradation, or regulate the expression of corresponding histone genes.

The close relationship between histone chaperones and histone degradation could be illustrated by the example of the NASP chaperone. Indeed it has been shown that NASP buffers the amount of free H3 and H4 and fine-tunes the cellular amount of these histones by controlling their degradation by autophagy.[24]

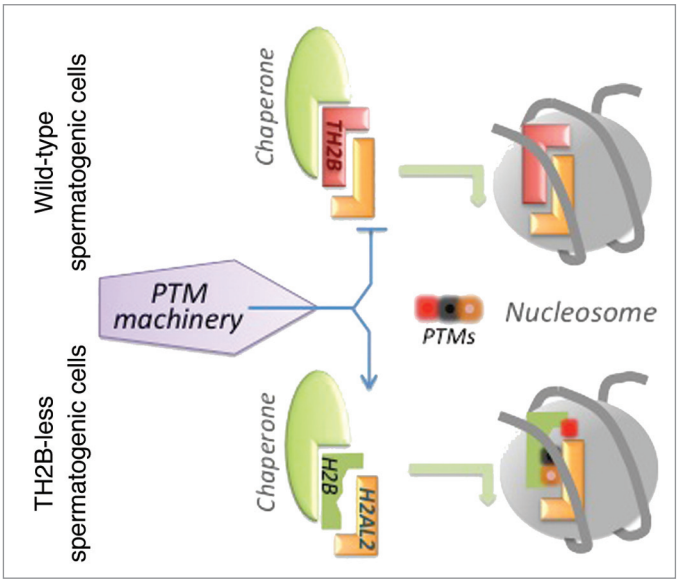

Figure 2. A model for variant-dependent histone modifications by the PTM machinery within chaperones. The example of the TH2B-H2AL2 dimer is used to illustrate the hypothesis that the forced association of H2AL2 with H2B instead of TH2B, would render certain H2B positions accessible to the PTM machinery.

The most interesting scenario with respect to this Extra View, concerns a potential regulation of transcription mediated by histone chaperones. There are examples in the recent literature that support this hypothesis. The known transcriptional regulator Spt2 has been identified as a novel histone chaperone that could be involved in coupling transcriptional activity and histone assembly in nucleoli.[25] Another example of a chaperone-controlled transcription has very recently been reported following the recruitment of HIRA and deposition of H3.3 at the site of DNA damage that bookmarks chromatin for a delayed recovery of transcription (positive regulation).[26] Finally, H3.3 and Hira have been proposed to recruit the PRC2 complex and to target the promoter of developmentally regulated genes (negative regulation).[27]

It is therefore possible to propose that a TH2B-loaded chaperone could specifically recruit a repressor machinery to the histone *h2b* gene cluster hence establishing a cross talk between a newly expressed histone variant and the gene cluster encoding its canonical counterparts (**Fig. 1**). An issue with this hypothesis is that it is not clear how the chaperones would be targeted to the *h2b* genes cluster. The specific chaperones for TH2B and H1T still await to be identified. Indeed their identification would allow more insights into the underlying mechanisms.

Cross-talk Between Histones: Functional Compensation

The generation of mice totally lacking TH2B in their spermatogenic cells surprisingly showed no defects in spermatogenesis.[13] Since TH2B globally replaces its somatic counterparts in early meiotic cells, it implies that a compensation for *h2b* expression is necessary to ensure normal chromatin functions. As mentioned above, a compensatory accumulation of H2B occurs in the absence of TH2B. However this observation is puzzling, since it could suggest that TH2B would have no specific role and that H2B could perfectly ensure all the putative activities of TH2B. A detailed analysis of the chromatin purified from spermatogenic cells lacking TH2B shed a new light on the real nature of the compensation by H2B. Indeed, a quantitative mass spectrometry approach, comparing histone PTMs in TH2B-less spermatogenic cells with those of wild type cells, revealed a surprisingly fine regulatory mechanism. Indeed, in TH2B-less spermatogenic cells, we found that specific PTMs specifically affect a number of key architectural positions within histones with nucleosome destabilization functions. Indeed, a total of 8 amino acids, in H4, H3 and H2B, showed enhanced PTMs and all were located in the histone fold parts of the corresponding histones. Two crotonylated lysines, H4K77 and H3K122, affect histone-DNA contact, while five methylated arginines on H4 and H2B, H4R35, H4R55, H4R67, and H2BR72, weaken histone-histone interactions.

All these modifications occur in the histone fold regions normally present in the heart of a nucleosome. This observation raises an important question regarding how the histone PTM machinery could have access to these residues buried within the nucleosome. Moreover, how would compensatory mechanisms specifically recognize and target these "strategic" amino acids, whose modification could affect the stability of a nucleosome? Modifying these residues after histone loading on chromatin would certainly require the dismantlement of nucleosomes. One hypothesis is that the histones could actually be modified at these sites before nucleosome assembly. This scenario would imply that histones are being modified while chaperoned and before their incorporation into chromatin (**Fig. 2**). If so, then in the context of the observation presented here, how could the absence of TH2B induce an enhanced histone PTMs on chaperone-associated histones?

An answer could come from our previous investigations showing that TH2B preferentially associates with the histone variant H2AL2. H2AL2 is a testis-specific late-expressing H2A variant that is expressed in spermatids prior to the replacement of histones by transition proteins.[28] In the absence of TH2B, the association of H2B with H2AL2 could expose specific residues, which would become accessible to histone PTM machineries, whereas they would normally be hidden within the TH2B-H2AL2 or H2B-H2A dimers (**Fig. 2**).

The reason why PTM modifications are also affecting H3 and H4 in the absence of TH2B is yet unclear. A possible answer would be that they could be accessed by the histone PTM

machinery during their assembly with the already modified H2A-H2B counterparts, prior to their incorporation.

Although these hypotheses are highly speculative, the literature now presents considerable body of reports uncovering a role for chaperones in modulating chromatin marks (for review see ref. 29). In general, chaperone-mediated histone modifications would be very important for the modification of several already characterized nucleosome-destabilizing histone PTMs such as H3K56 and H3K122 acetylations.[6] At least in the case of H3K56, the involvement of chaperones has clearly been demonstrated.[29]

We can therefore propose a critical role for the histone PTM machinery, which, by modifying histones within chaperones, would establish compensatory actions on histones in the absence of a particular variant (**Fig. 2**).

Conclusions

The spermatogenic cell response to the lack of TH2B has led to the discovery of an exquisite compensatory mechanism demonstrating both (1) the ability of *h2b* genes to sense the lack of specific H2B species and (2) the ability of the cells to use functional compensation mechanisms. The discussion here presents the idea that histone chaperones could mediate both responses. Histone chaperones could also mediate a "quality sensing" activity on chromatin to maintain normal chromatin functions under various potentially deleterious situations, including histone under-dosage and modifications of the nucleosomes stability parameters. In spermatogenic cells, in the absence of TH2B, these sensing mechanisms are efficient enough to fully rescue spermatogenesis, allowing the production of fertile sperm cells. Similar mechanisms are also very probably involved in the rescue of spermatogenesis in the absence of H1T.

Yet, although the hypothesis of "chromatin quality sensing" mechanisms driven by histone chaperones is attractive, specific approaches remain to be implemented to validate and elucidate the underlying molecular details.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Rathke C, Baarends WM, Awe S, Renkawitz-Pohl R. Chromatin dynamics during spermiogenesis. Biochim Biophys Acta 2013; PMID:24091090
2. Gaucher J, Reynoird N, Montellier E, Boussouar F, Rousseaux S, Khochbin S. From meiosis to postmeiotic events: the secrets of histone disappearance. FEBS J 2010; 277:599-604; PMID:20015078; http://dx.doi.org/10.1111/j.1742-4658.2009.07504.x
3. Montellier E, Rousseaux S, Zhao Y, Khochbin S. Histone crotonylation specifically marks the haploid male germ cell gene expression program: post-meiotic male-specific gene expression. Bioessays 2012; 34:187-93; PMID:22170506; http://dx.doi.org/10.1002/bies.201100141
4. Bönisch C, Hake SB. Histone H2A variants in nucleosomes and chromatin: more or less stable? Nucleic Acids Res 2012; 40:10719-41; PMID:23002134; http://dx.doi.org/10.1093/nar/gks865
5. Talbert PB, Henikoff S. Histone variants--ancient wrap artists of the epigenome. Nat Rev Mol Cell Biol 2010; 11:264-75; PMID:20197778; http://dx.doi.org/10.1038/nrm2861
6. Tropberger P, Schneider R. Scratching the (lateral) surface of chromatin regulation by histone modifications. Nat Struct Mol Biol 2013; 20:657-61; PMID:23739170; http://dx.doi.org/10.1038/nsmb.2581
7. Turner JMA. Meiotic sex chromosome inactivation. Development 2007; 134:1823-31; PMID:17329371; http://dx.doi.org/10.1242/dev.000018
8. Ichijima Y, Sin H-S, Namekawa SH. Sex chromosome inactivation in germ cells: emerging roles of DNA damage response pathways. Cell Mol Life Sci 2012; 69:2559-72; PMID:22382926; http://dx.doi.org/10.1007/s00018-012-0941-5
9. Mueller JL, Mahadevaiah SK, Park PJ, Warburton PE, Page DC, Turner JMA. The mouse X chromosome is enriched for multicopy testis genes showing postmeiotic expression. Nat Genet 2008; 40:794-9; PMID:18454149; http://dx.doi.org/10.1038/ng.126
10. Gaucher J, Boussouar F, Montellier E, Curtet S, Buchou T, Bertrand S, Hery P, Jounier S, Depaux A, Vitte A-L, et al. Bromodomain-dependent stage-specific male genome programming by Brdt. EMBO J 2012; 31:3809-20; PMID:22922464; http://dx.doi.org/10.1038/emboj.2012.233
11. Boussouar F, Rousseaux S, Khochbin S. A new insight into male genome reprogramming by histone variants and histone code. Cell Cycle 2008; 7:3499-502; PMID:19001855; http://dx.doi.org/10.4161/cc.7.22.6975
12. Govin J, Caron C, Lestrat C, Rousseaux S, Khochbin S. The role of histones in chromatin remodelling during mammalian spermiogenesis. Eur J Biochem 2004; 271:3459-69; PMID:15317581; http://dx.doi.org/10.1111/j.1432-1033.2004.04266.x
13. Montellier E, Boussouar F, Rousseaux S, Zhang K, Buchou T, Fenaille F, Shiota H, Debernardi A, Héry P, Curtet S, et al. Chromatin-to-nucleoprotamine transition is controlled by the histone H2B variant TH2B. Genes Dev 2013; 27:1680-92; PMID:23884607; http://dx.doi.org/10.1101/gad.220095.113
14. Khochbin S. Histone H1 diversity: bridging regulatory signals to linker histone function. Gene 2001; 271:1-12; PMID:11410360; http://dx.doi.org/10.1016/S0378-1119(01)00495-4
15. Lin Q, Sirotkin A, Skoultchi AI. Normal spermatogenesis in mice lacking the testis-specific linker histone H1t. Mol Cell Biol 2000; 20:2122-8; PMID:10688658; http://dx.doi.org/10.1128/MCB.20.6.2122-2128.2000
16. Drabent B, Saftig P, Bode C, Doenecke D. Spermatogenesis proceeds normally in mice without linker histone H1t. Histochem Cell Biol 2000; 113:433-42; PMID:10933220
17. Fantz DA, Hatfield WR, Horvath G, Kistler MK, Kistler WS. Mice with a targeted disruption of the H1t gene are fertile and undergo normal changes in structural chromosomal proteins during spermiogenesis. Biol Reprod 2001; 64:425-31; PMID:11159343; http://dx.doi.org/10.1095/biolreprod64.2.425
18. Gunjan A, Verreault A. A Rad53 kinase-dependent surveillance mechanism that regulates histone protein levels in S. cerevisiae. Cell 2003; 115:537-49; PMID:14651846; http://dx.doi.org/10.1016/S0092-8674(03)00896-1
19. Singh RK, Kabbaj M-HM, Paik J, Gunjan A. Histone levels are regulated by phosphorylation and ubiquitylation-dependent proteolysis. Nat Cell Biol 2009; 11:925-33; PMID:19578373; http://dx.doi.org/10.1038/ncb1903
20. Burgess RJ, Zhang Z. Histone chaperones in nucleosome assembly and human disease. Nat Struct Mol Biol 2013; 20:14-22; PMID:23288364; http://dx.doi.org/10.1038/nsmb.2461
21. Bonner WM, Wu RS, Panusz HT, Muneses C. Kinetics of accumulation and depletion of soluble newly synthesized histone in the reciprocal regulation of histone and DNA synthesis. Biochemistry 1988; 27:6542-50; PMID:3146349; http://dx.doi.org/10.1021/bi00417a052
22. Oliver D, Granner D, Chalkley R. Identification of a distinction between cytoplasmic histone synthesis and subsequent histone deposition within the nucleus. Biochemistry 1974; 13:746-9; PMID:4359465; http://dx.doi.org/10.1021/bi00701a017
23. Gunjan A, Paik J, Verreault A. The emergence of regulated histone proteolysis. Curr Opin Genet Dev 2006; 16:112-8; PMID:16510276; http://dx.doi.org/10.1016/j.gde.2006.02.010
24. Cook AJL, Gurard-Levin ZA, Vassias I, Almouzni G. A specific function for the histone chaperone NASP to fine-tune a reservoir of soluble H3-H4 in the histone supply chain. Mol Cell 2011; 44:918-27; PMID:22195965; http://dx.doi.org/10.1016/j.molcel.2011.11.021
25. Osakabe A, Tachiwana H, Takaku M, Hori T, Obuse C, Kimura H, Fukagawa T, Kurumizaka H. Vertebrate Spt2 is a novel nucleolar histone chaperone that assists in ribosomal DNA transcription. J Cell Sci 2013; 126:1323-32; PMID:23378026; http://dx.doi.org/10.1242/jcs.112623

26. Adam S, Polo SE, Almouzni G. Transcription recovery after DNA damage requires chromatin priming by the H3.3 histone chaperone HIRA. Cell 2013; 155:94-106; PMID:24074863; http://dx.doi.org/10.1016/j.cell.2013.08.029
27. Banaszynski LA, Wen D, Dewell S, Whitcomb SJ, Lin M, Diaz N, Elsässer SJ, Chapgier A, Goldberg AD, Canaani E, et al. Hira-dependent histone H3.3 deposition facilitates PRC2 recruitment at developmental loci in ES cells. Cell 2013; 155:107-20; PMID:24074864; http://dx.doi.org/10.1016/j.cell.2013.08.061
28. Govin J, Escoffier E, Rousseaux S, Kuhn L, Ferro M, Thévenon J, Catena R, Davidson I, Garin J, Khochbin S, et al. Pericentric heterochromatin reprogramming by new histone variants during mouse spermiogenesis. J Cell Biol 2007; 176:283-94; PMID:17261847; http://dx.doi.org/10.1083/jcb.200604141
29. Avvakumov N, Nourani A, Côté J. Histone chaperones: modulators of chromatin marks. Mol Cell 2011; 41:502-14; PMID:21362547; http://dx.doi.org/10.1016/j.molcel.2011.02.013